European Journal of Medicinal Chemistry 83 (2014) 534–546

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Aryl-1,3,5-triazine derivatives as histamine H_4 receptor ligands

Dorota Łażewska [a], Małgorzata Więcek [a], Joanna Ner [a], Katarzyna Kamińska [a], Tim Kottke [b], J. Stephan Schwed [b], Małgorzata Zygmunt [c], Tadeusz Karcz [a], Agnieszka Olejarz [a], Kamil Kuder [a], Gniewomir Latacz [a], Marek Grosicki [a], Jacek Sapa [c], Janina Karolak-Wojciechowska [d], Holger Stark [b, e], Katarzyna Kieć-Kononowicz [a, *]

[a] Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Medyczna 9 Str., 30-688 Kraków, Poland
[b] Goethe University, Institute of Pharmaceutical Chemistry, Biozentrum, ZAFES/CMP/ICNF, Max-von-Laue-Str. 9, 60438 Frankfurt am Main, Germany
[c] Department of Pharmacodynamics, Jagiellonian University Medical College, Medyczna 9 Str., 30-688 Kraków, Poland
[d] Institute of General and Ecological Chemistry, Technical University of Łódź, Żeromskiego 116 Str., 90-924 Łódź, Poland
[e] Heinrich-Heine-University, Institute of Pharmaceutical and Medicinal Chemistry, Universitaetsstr. 1, 40225 Duesseldorf, Germany

ARTICLE INFO

Article history:
Received 25 January 2014
Received in revised form
17 April 2014
Accepted 16 June 2014
Available online 17 June 2014

Keywords:
Histamine H_4 receptor
4-Methylpiperazines
2,4,6-Trisubstituted 1,3,5-triazines
Anti-inflammatory properties

ABSTRACT

A series of novel 2-amino-4-(4-methylpiperazin-1-yl)-1,3,5-triazine derivatives with different aryl substituents in the 6-position was designed, synthesized and evaluated for histamine H_4 receptor (H_4R) affinity in Sf9 cells expressing human H_4R co-expressed with G-protein subunits. Triazine derivative **8** with a 6-(*p*-chlorophenyl) substituent showed the highest affinity with hH_4R K_i value of 203 nM and was classified as an antagonist in cAMP accumulation assay. This compound, identified as a new lead structure, demonstrated also anti-inflammatory properties in preliminary studies in mice (carrageenan-induced edema test) and neither possessed significant antiproliferative activity, nor modulated CYP3A4 activity up to concentration of 25 μM. In order to discuss structure–activity relationships molecular modeling and docking studies were undertaken.

1. Introduction

The human histamine H_4 receptor (hH_4R) was cloned in 2000 and has constituted an interesting target for drug development thereafter. Pharmacological studies suggest utility of H_4R antagonists/inverse agonists in the treatment of inflammatory and immunomodulatory diseases, e.g., allergic rhinitis, asthma, atopic dermatitis, colitis and pruritus [1]. Among described H_4R antagonists/inverse agonists pyrimidine-2-amine derivatives consist important lead structures with many potent compounds [for review see [2–7]]. Some of early developments already made their way to clinical trials and promising results from the clinical studies for compounds **UR-63325** and **ZPL-38937887** were reported [8,9].

The 1,3,5-triazine (*s*-triazine) moiety is present in many biologically active structures with the broad range of therapeutic utility. Such compounds have been proved to exhibit antimicrobial [10,11], antimalarial activity [12] and chemotherapeutic potential [13,14]. Moreover, for some triazine dimers the ability to mimic the protein A binding to murine and human IgG antibody was described [15]. The chemistry of 1,3,5-triazine was recently reviewed by Blotny [16].

A couple of 4-(4-methylpiperazin-1-yl)-1,3,5-triazines were disclosed in the patent application from Johnson&Johnson and some of them showed high affinities (K_i < 20 nM) for hH_4R (e.g. **1**, Fig. 1) [17].

Taking together the published results on pyrimidine derivatives (e.g. **2**) [18] as well as information on triazines (e.g. **1**) [17] a search was initiated to find active compounds among 2-amino-4-(4-methylpiperazin-1-yl)-1,3,5-triazines. In this work, the synthesis, H_4R affinities, functional properties, receptor selectivity and anti-inflammatory properties of these compounds (exemplified by general structure on Fig. 2) are described. Also for selected compounds antiproliferative effect and metabolic stability was checked.

2. Results and discussion

2.1. Chemistry

The general synthetic procedure for preparation of 2,4,6-trisubstituted 1,3,5-triazines is outlined in Scheme 1. Triazine derivatives **3**–**23** were obtained as the result of the reaction of esters

* Corresponding author.
E-mail address: mfkonono@cyf-kr.edu.pl (K. Kieć-Kononowicz).

http://dx.doi.org/10.1016/j.ejmech.2014.06.032
0223-5234/

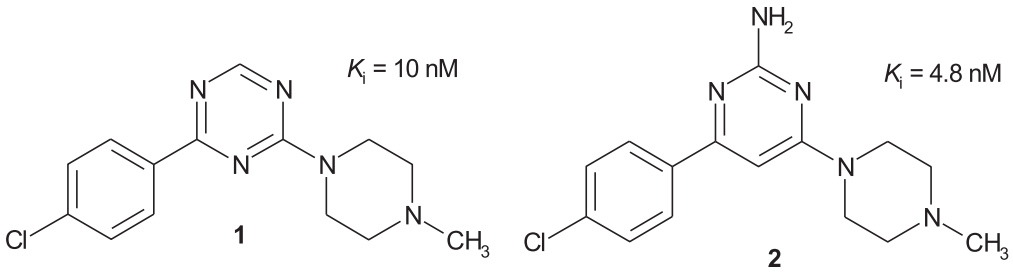


Fig. 1. Structures and H_4R affinities of (4-methylpiperazin-1-yl)-1,3,5-triazine (**1** [17]) and -pyrimidine-2-amine derivatives (**2** [18]).

3a–**23a** with biguanidine dihydrochloride. 4-Methylpiperazin-1-yl biguanidine dihydrochloride intermediate was obtained by the heating of cyanoguanide and 4-methylpiperazine dihydrochloride in 1-butanol [19]. All acid methyl esters were commercially available (except **7a** and **21a** which were synthesized according to the known procedure [20]). Cyclization of 4-methylpiperazin-1-yl biguanidine dihydrochloride with the appropriate carboxylic acid methyl ester in the presence of sodium methoxide have been known to yield 1,3,5-triazines [21]. The reaction was led in the boiling point and proceeded in low yields (4–35%).

2.2. Histamine H_4 receptor screening

The series of novel substituted phenyl derivatives of 1,3,5-triazine with one to three substituents at the benzene ring was screened at hH_4R in [^{3}H]histamine binding experiments (Table 1). Investigated was the SAR of the 6 substituent of the 1,3,5-triazine derivatives on the hH_4R activity. All compounds share the 4-methylpiperazinyl moiety (as basic center) and the primary aromatic amino group as additional basic center as well as hydrogen acceptor/donor functionality. These both elements are widely present in many published H_4R antagonists [2–7]. Most of the obtained compounds showed micro/submicromolar affinities.

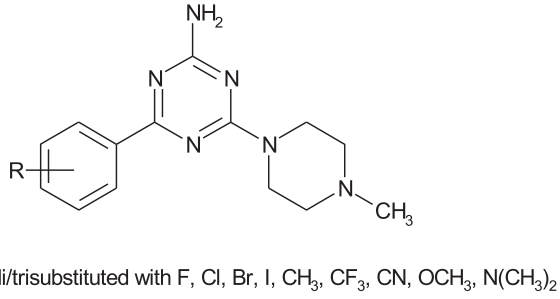
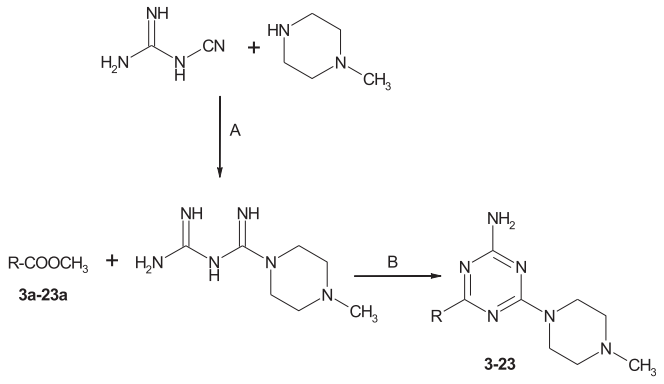


Fig. 2. General structure of synthesized compounds.



Scheme 1. Synthetic route leading to 2,4,6-trisubstituted 1,3,5-triazines. Reagents and conditions: (A) BuOH, temperature gradually increased from 50 to 90 °C during 1 h, 5 h reflux; (B) MeONa, reflux from 15 to 30 h.

The most potent in these group was compound **8** with chlorine substituent in the *para*-position with submicromolar affinity (K_i = 203 nM [6]). The change of chlorine position to *meta* (**5**, K_i = 408 nM) and especially to *ortho* (**4**, K_i = 1261 nM) position drastically reduced affinity. Generally, the same trend was also observed for methyl and cyano substituents (compare **6** vs **12** and **7** vs **14**). In addition, the change of chlorine in the *para*-position for different substituents (F, Br, I, CH_3, CF_3, CN, $N(CH_3)_2$, compounds **9**–**15**), led to decrease of potency compared with that of **8**. Interestingly, compound **3**, the unsubstituted analogue, had higher affinity than the majority of the substituted derivatives.

The introduction of the second substituent (chlorine **18** or methoxy group **16**, **17**) to *meta*-position e.g. compound **5** did not improve the affinity. Two fluorine substituents in 2- and 6-position (**19**) are less beneficial than two (**20**) or three atoms (**21**) in respectively the 3,5-position and the 3,4,5-position.

Comparing the present results with the published data for pyrimidine analog of **8** (compound **2**, Fig. 1 [18]) a reduction in affinity of more than one log unit was noticed. Although, it seems that the additional nitrogen in the central core (triazine ring instead of pyrimidine one) is detrimental for H_4R affinity, some compounds display submicromolar affinities. So further investigations should be done (e.g. blocking free amino group) as some related triazines with high H_4R affinities have recently been described [17].

2.3. Functional properties at histamine H_4 receptor and selectivity over histamine H_3 receptor for selected compounds

In order to better characterize the pharmacological profile of the investigated compounds selected structures (**8** and **10**) were additionally tested for their functional properties in cAMP accumulation assay at recombinant cellular model. Their selectivity over histamine H_3 receptor (H_3R) subtype, representing the highest degree of homology with H_4R, was also evaluated.

Investigated compounds caused a blockade of the histamine-induced cAMP reduction in CHO-hH$_4$R-cAMPzen cells, co-treated with forskolin and were therefore classified as antagonists at H_4Rs. Antagonist potency (IC_{50}) was determined by performing a dose response curve in presence of H_4R agonist (histamine, 140 nM, corresponding to its EC_{80}) and forskolin (10 μM) (Fig. 3). Obtained IC_{50} values for both evaluated compounds were well in accordance with their K_i values at hH_4Rs and the order of potency also was retained in functional tests (**8**, K_i = 203 nM, IC_{50} = 512 nM; **10**, K_i = 524 nM, IC_{50} = 1630 nM).

Since H_4R shows the highest sequence homology (~60% in the transmembrane domains) to the H_3R subtype [22], considered structures were additionally tested for their interaction with H_3R.